Designation: E 1054 – 91

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Standard Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[1]

This standard is issued under the fixed designation E 1054; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These practices are designed to determine the ability of chemical inactivators (neutralizers) to interrupt the killing action of antimicrobial agents found in disinfectants, sanitizers and antiseptics.

NOTE 1—A knowledge of microbiological techniques is required for these procedures.

1.2 *This standard does not purport to address the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Summary of Practices

2.1 These practices describe techniques to determine:

2.1.1 The maximum concentration of chemical inactivator that is tolerated by the test microorganism(s), and

2.1.2 The interruption of the killing action of the antimicrobial by the neutralizer.

2.2 The techniques should be performed sequentially as described.

3. Significance and Use

3.1 In disinfectant, sanitizer, and antiseptic tests, or in the testing of preserved products, antimicrobial agents carried over by the mechanical manipulations of the given test must be adequately inactivated (neutralized). Inadequate neutralization allows the continued kill of cells in the subculture or neutralizer system or prevents the growth of surviving cells and results in false interpretation of the data.

3.2 Several chemicals such as lecithin, polysorbate 80, and sodium thiosulfate, for example, chemically react with certain antimicrobials such that the antimicrobial activity is negated. The concentration and type of inactivator necessary to completely interrupt activity of a particular antimicrobial may be determined by following these conceptual approaches.

4. Apparatus, Materials, and Reagents

4.1 Because the types of apparatus, materials and reagents required for the various antimicrobial tests or testing preserved products are so diverse, it is impractical to list these items in these practices. Standard bacteriological equipment for performance of the particular tests should be used.

5. Conceptual Aspects

5.1 Inactivator studies must be validated with the target organisms which are specified for the particular disinfectant, sanitizer, or antiseptic test method. There are two issues of concern; namely, the neutralizer system must not kill the target organism and the neutralizer system must inactivate the antimicrobial.

6. Test for Maximum Tolerated Concentration (MTC) of Neutralizer

6.1 Validation of the nonantimicrobial nature of an inactivator should conform to the mechanical aspects (volumes, glassware, temperatures, and so forth) of the specific test protocol.

6.2 The suitability of using a particular buffered peptone water or other fluid as a base for a neutralizer system should be determined. The target organism should be added to the buffered peptone water at a level which is determined to be the maximum acceptable level after biocide treatment in the particular test. Microbial counts should be determined at time zero and at 30 min contact time. The extent of replication should be considered (see Section 8). The counts between these two times should not differ significantly since the buffered peptone collection fluid should be expected to not cause a decrease in microbial survival.

6.3 The intended neutralizer can then be added to the buffered peptone system at varying concentrations. The effect of each concentration on the target organism after 30 min contact time should be compared to the 30 min contact time of the control (no neutralizer). Select the maximum concentration of neutralizer which does not significantly reduce the counts of the target organism. The extent of replication should be considered (see Section 8).

NOTE 2—The MTC of neutralizer may differ depending on the target organism.

7. Test for the Effect of the MTC on the Antimicrobial

7.1 The effects of the MTC of the neutralizer are then tested by adding specific concentrations of the antimicrobial to the MTC system. Target organisms are added to the neutralizer systems with and without antimicrobial agents and comparisons of microbial counts are made after 30 min contact times. Counts of the neutralizer-antimicrobial system should not differ significantly from the counts of the control. The extent of replication should be considered (see Section 8).

[1] These practices are under the jurisdiction of ASTM Committee E-35 on Pesticides and are the direct responsibility of Subcommittee E35.15 on Antibacterial and Antiviral Agents.

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8. Precision and Interpretation of Data

8.1 Plate count data from each treatment and its corresponding control are transformed to square root values. The mean differences are tested for significance by the *t* test.[2,3]

9. Keywords

9.1 antimicrobial; antiseptic; disinfectant; inactivator; neutralizer; preservative; sanitizer

[2] Barker, T. B., "Quality by Experimental Design," *Vol 4—Quality and Reliability*, Marcel Dekker, Inc., 270 Madison Ave., New York, NY, pp 141–147, 1985.

[3] Niemela, S., *Statistical Evaluation of Results from Quantitative Microbiological Examinations*, Report No. 1, 2nd Edition, Nordisk Metodik-Kommitte For Livsmedel, c/o Statens Livsmedelsverk, Box 622, S-75126 Uppsala, Sverige, p 19, 1983.

Designation: E 1115 – 91

Standard Test Method for Evaluation of Surgical Hand Scrub Formulations[1]

This standard is issued under the fixed designation E 1115; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to evaluate antimicrobial agents in formulations for utility and effectiveness as surgical hand scrubs. It is intended for determining both immediate microbial reductions and reductions with regular use (residual effects).

NOTE 1—A knowledge of microbiological techniques is required for these procedures.

1.2 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standard:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[2]

2.2 *Other Documents:*

Standard Method for the Examination of Dairy Products[3]

AATCC Method 90-1965[4]

3. Summary of Test Method

3.1 This test method is conducted on panelists selected from a group of volunteers who have refrained from using any antimicrobials for at least two weeks prior to initiation of the test. At least twelve panelists are selected from this group on the basis of high initial bacteria count, 1×10^5 per hand as determined by baseline measurements of the bacteria on their hands.

3.2 The selected panelists perform a simulated surgical scrub under the supervision of an individual competent in aseptic technique. One-third of the panelists' hands are sampled immediately after the scrub (within 5 min), one-third after 3 h and the remaining hands, 6 h after scrubbing. No more than one hand of a panelist is sampled at a given time interval.

3.3 Ten additional scrubs are performed with the test formulation over a 5-day period following the initial scrub. The hands are sampled two additional times, once after the second scheduled use of the product and again after the last scheduled scrub.

4. Significance and Use

4.1 The procedure in this test method should be used to evaluate the ability of a test formulation to reduce the bacterial population of the hands immediately after a single and multiple use and to determine the trend in growth over a 6-h period after single and multiple usages.

5. Apparatus

5.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter.

5.2 *Incubator*—Any incubator capable of maintaining a temperature of 30 ± 2°C may be used.

5.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterility is acceptable.

5.4 *Timer (stop-clock)*, that can be read for minutes and seconds.

5.5 *Hand Washing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists.

5.5.1 *Water Faucet(s)*, to be located above the sink at a height that permits the hands to be held higher than the elbows during the washing procedure. (It is desirable for the height of the faucet(s) to be adjustable.)

5.6 *Tap Water Temperature Regulator and Temperature Monitor*, to monitor and regulate water temperature to 40 ± 2°C.

6. Materials and Reagents

6.1 *Petri Dishes*—100 by 15 mm. Required for performing standard plate count.[5]

6.2 *Bacteriological Pipets*, 10.0 and 2.2 or 1.1-mL capacity.[6]

6.3 *Water-Dilution Bottles*—Any sterilizable glass container having a 150 to 200-mL capacity and tight closures may be used.[7]

6.4 *Baseline Control Soap*—A liquid castile soap or other liquid soap containing no antimicrobial.

6.5 *Gloves*—Sterile loose fitting gloves of latex, unlined,

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[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

[3] Available from American Public Health Association, Inc., Washington, DC, pter: Standard Plate Count Method.

[4] *AATCC Test Methods*, 1968 Technical Manual, Section B-175, available from the American Association of Textile Chemists and Colorists, P.O. Box 12215, Research Triangle Park, NC 27709.

[5] Presterilized/disposable plastic petri dishes are available from most local laboratory supply houses.

[6] Presterilized/disposable bacteriological pipets are available from most local laboratory supply houses.

[7] Dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

possessing no antimicrobial properties.[8]

6.6 *Test Formulation*—Directions for use of test formulation should be included if available. If none are available, use directions provided in this test method (see Section 11).

6.7 *Sampling Solution*[9]—Dissolved 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isooctylphenoxypolyethoxyethanol[10] in 1 L distilled water. Adjust to pH 7.8. Dispense in 75 mL volumes into water dilution bottles, or other suitable containers, and sterilize for 20 min at 121°C. Include an antimicrobial inactivator specific for the test formulation being evaluated in the sampling solution used to collect the bacterial samples from the hand following the final wash with the test formulation.

6.8 *IMPORTANT*—A definitive recommendation regarding the inclusion of an inactivator in sampling solution (6.7) used for bacterial collections prior to the final wash can not be made. The following two points should be considered in making a decision: (*1*) If an inactivator is included in the sampling solution used prior to the final wash, will residual inactivator on the skin reduce the efficacy of the test formulation in subsequent washes and result in higher than expected bacterial counts? (*2*) Can samples collected without an inactivator be processed quickly enough to avoid decreased bacterial count due to continued action of the test formulation? Whatever the decision, to facilitate the comparison of results across studies, the investigator should indicate whether or not an inactivator has been included.

6.9 *Dilution Fluid*—Butterfield's[11] phosphate buffered water adjusted to pH 7.2 and containing an antimicrobial inactivator specific for the test formulation.

6.10 *Soybean-casein Digest Agar*[12], with supplemental polysorbate 80 (0.5 to 10 g/L) to stimulate growth of lipophilic organisms.

6.11 *Fingernail Cleaning Sticks*, such as Pre-Op® Premium Nail Cleaner.[13]

6.12 *Sterile Hand Scrub Brushes*[14] (required only if specified for use with test formulation).

7. Test Panelists

7.1 Panelists shall consist of healthy adult volunteers who have no clinical evidence of dermatosis, have not received antibiotics or taken oral contraceptives two weeks prior to the test, and who agree to abstain from these materials until the conclusion of the test.

8. Preparation of Volunteers

8.1 At least two weeks prior to start of the test, enroll approximately 20 volunteers as potential test subjects.

8.2 Instruct the volunteers to avoid contact with antimicrobials (other than the test formulation) for the duration of the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, soaps, and materials such as acids, bases, and solvents. Bathing in chlorinated pools and hot tubs is to be avoided. Volunteers are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobials can not be avoided.

9. Procedure

9.1 After panelists have refrained from using antimicrobials for at least two weeks, perform wash with baseline control soap (see 5.4, and Section 10). Volunteers are not to have washed their hands on this day 2 h prior to baseline determination. After washing, determine first estimate of baseline bacterial population by sampling hands and enumerating the bacteria in the sampling solution. This is Day 1 of "Baseline Period." Repeat this baseline determination procedure on Days 3 and 7, Days 3 and 5, or Days 5 and 7 of "Baseline Period" to obtain three estimates of baseline population. After obtaining the first and second estimates of the baseline populations, select, as panelists, at least twelve volunteers who exhibited at each sampling intervals, counts 1×10^5. The three estimates of the baseline population, obtained for each of the twelve selected subjects, are averaged to obtain the mean baseline counts.

9.2 A basic random sampling plan should be followed. The number of panelists and sampling times depend on the test formulation but must establish the onset and extent of the bacterial suppression and the duration of suppression below the baseline counts. Equal numbers of panelists should be assigned for sampling time, drug and handedness. A typical balanced randomization plan for testing a block of panelists follows:

Panelists No.	Post Scrub Sampling Time, hour		
	0-h	3-h	6-h
1	left hand	right hand	
2	left hand		right hand
3	right hand	left hand	
4	right hand		left hand
5		left hand	right hand
6		right hand	left hand

9.2.1 The number of panelists per block may vary but must be devisable by two and by the number of sampling times in order to assign equal number of left and right hands to each sampling time.

9.2.2 The minimum number of panelists depend on variability encountered in the study and relative efficacy of drugs. Use of less than twelve panelists each per drug is not advised for final product evaluations. In using larger numbers of panelists, it is only necessary to increase the number of balanced blocks.

9.3 No sooner than 12 h, nor longer than 4 days after completion of their baseline determination, panelists perform initial scrub with the test formulation. Determine, according to the random sampling plan, bacterial populations on the panelists' hands at the assigned sampling interval (0 h, 3 h, 6 h) after scrubbing. Determine bacterial

[8] A suitable glove, Pharmaseal® 8873C, (sterile) Flexam Latex Procedure Glove from American Pharmaseal Laboratories, Glendale, CA 91209. A zone of inhibition test such as AATCC Test Method 90-1965 may be used to evaluate antimicrobial properties of gloves.

[9] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130.

[10] Triton X-100, available from Rohm and Haas Co., Philadelphia, PA.

[11] Butterfield's Phosphate Buffer, *Journal of the Association of Official Agricultural Chemist*, Vol 27, 1939, p. 625.

[12] *United States Pharmacopeia*, XX: United States Pharmacopeial Convention, Inc., Rockville, MD. Chapter: Microbial Limit Test.

[13] Pre-Op® Premium Nail Cleaner (Plastic), Product No. 8014-12, Manufactured by Davis and Geck Laboratory, One Caster St., Danbury, CT 06813.

[14] A suitable brush, Hand Scrub Brush, Wood, No. 3390, is available from Graham Field Surgical Co., Inc., New Hyde Park, NY 11040.

population by sampling hands and enumerating the bacteria in the sampling solution as specified in Sections 13 and 14. Repeat this scrubbing and sampling procedure the next day (Day 2). On Day 5, repeat the sampling procedure after scrubbing with the test material two additional times on Day 2 and three times per day on Day 3 and Day 4 with at least a 1-h interval between scrubs. Perform one scrub on Day 5 prior to sampling. In summary, the panelists scrub a total of eleven times with the test formulation, once on Day 1 and Day 5 and three times per day on Days 2, 3, and 4. Collect bacterial samples following three of the eleven scrubs. Collect the samples following the single scrubs on Days 1 and 5 and following the first scrub on Day 2. This mimics typical usage and permits determination of both immediate and longer-term reductions.

10. Washing Technique for Baseline Determinations

10.1 Volunteers clean under fingernails with nail stick and clip fingernails to ≤2-mm free edge. Remove all jewelry from hands and arms.

10.2 Rinse hands including two thirds of forearm under running tap water 38 to 42°C for 30 s. Maintain hands higher than elbows during this procedure and steps outlined in 10.3, 10.4, and 10.5.

10.3 Wash hands and forearms with baseline control soap for 30 s using water as required to develop lather.

10.4 Rinse hands and forearms, thoroughly removing all lather, for 30 s under tap water.

10.5 Don rubber gloves (6.5) used in sampling hands and secure gloves at wrist.

11. Surgical Scrub Technique to Be Used Prior to Bacterial Sampling

11.1 Repeat 10.1 and 10.2.

11.2 Perform surgical scrub with test formulation in accordance with directions furnished with formulation.

NOTE 2—If no instructions are provided with the test formulation, use the 10-min scrub procedure in 11.3.

11.3 *Ten-Minute Scrub Procedure:*

11.3.1 Dispense formulation into hands.

11.3.2 Set and start timer for 5 min (time required for the steps in 11.3.3 through 11.3.7).

11.3.3 With hands, distribute formulation over hands and lower two thirds of forearms.

11.3.4 If scrub brush is to be used, pick up with finger tips and pass under tap to wet without rinsing formulation from hands.

11.3.5 Alternatively scrub right hand and lower two thirds of forearm and left hand and lower two thirds of forearm.

11.3.6 Rinse both hands, the lower two thirds of forearms, and the brush for 30 s.

11.3.7 Place brush in sterile dish within easy reach.

11.3.8 Repeat 11.3.1 through 11.3.6 so that each hand and forearm is washed twice. The second wash and rinse should be limited to the lower one third of the forearms and the hands.

11.3.9 Perform final rinse. Rinse each hand and forearm separately for 1 min per hand.

11.3.10 Don rubber gloves (6.5) used in sampling hands and secure at wrist.

12. Surgical Scrub Technique When Bacterial Samples Are Not Indicated

12.1 Perform technique as described in Section 11, except omit 11.3.10. Panelists dry hands with clean paper towel after final rinse of hands.

13. Sampling Techniques

13.1 At specified sampling times, aseptically add 75 mL of sampling solution (6.7) to glove and hand to be sampled and occlude glove above wrist.

13.2 After adding sampling solution, uniformly massage all surfaces of hand for 1 min.

13.3 After massaging, aseptically sample the fluid of the glove.

14. Enumeration of Bacteria in Sampling Solution

14.1 Enumerate the bacteria in the sampling solution by a standard plate count procedure such as that described in Standard Methods for the Evaluation of Dairy Products[3] but using soybean-casein digest agar (6.9) and a suitable inactivator[15] for the antimicrobial where necessary. Prepare sample dilutions in dilution fluid (6.8). Plate in duplicate. Incubate plated sample at 30 ± 2°C for 48 h before reading.

15. Determination of Reduction Obtained

15.1 Determine at each sampling interval, changes from baseline counts obtained with test material.

15.2 For a more realistic appraisal of the activity of products, all raw data should be converted to common (base 10) logarithms. Reductions should be calculated from the average of the logarithms. This will also facilitate statistical analysis of data if desired.

16. Comparison of Test Materials With a Control Material

16.1 It may be desirable to compare the test material with a control material. If this is the case, an equivalent number of panelists should be assigned to the control product on a random basis. All test parameters will be equivalent for both products, although the scrub procedure for an established product may be different. Both products should be run concurrently. Identity of products used by panelists should be blinded from those counting plates and analyzing data. A suggested positive control is a surgical scrub formulation approved by the U.S. Food and Drug Administration.

16.2 Compare, at each sampling interval, changes from baseline counts obtained with test material to changes obtained with control material.

17. Precision and Bias

17.1 A precision and bias statement can not be made for this test method at this time.

18. Keywords

18.1 antimicrobial; efficacy; glove juice; surgical scrub

[15] If suitable inactivator for antimicrobial is not known, tests should be performed to determine appropriate neutralizer. A suitable test is described in Practices E 1054.

Designation: E 1173 – 93

Standard Test Method for Evaluation of a Pre-Operative Skin Preparation[1]

This standard is issued under the fixed designation E 1173; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The test method is designed to determine the ability of a pre-operative skin preparation to reduce the resident microbial flora on the skin when used in a skin pre-operative preparation procedure.

1.2 In this test method, metric units are used for all applications except for distance, in which case inches are used and metric units follow in parentheses.

NOTE 1—A knowledge of microbiological techniques is required for these procedures.

1.3 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[2]

3. Summary of Test Method

3.1 This test method is conducted on panelists selected from a group of volunteers who, after refraining from using topical and oral antimicrobials for at least two weeks, exhibit high skin flora counts on the abdomen and groin.

3.2 Activity of the pre-operative skin preparation is measured by comparing microbial counts obtained at various time intervals after application of the pre-operative treatment to skin sites located on the abdomen and in the groin to counts obtained from the same sites prior to treatment application.

NOTE 2—Microbial samples are collected a minimum of three times after treatment application. The first two collections are made 10 min and 30 min post treatment, and the third collection is made no less than 4 h post treatment but may be made later.

4. Significance and Use

4.1 The procedure should be used to test antimicrobial-containing preparations that are intended to be fast-acting and to significantly reduce the number of organisms on intact skin.

4.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[3]

5. Apparatus

5.1 *Colony Counter*—Any of several types may be used, for example, Quebec colony counter.

5.2 *Incubator*—Any incubator capable of maintaining a temperature of 30 ± 2°C may be used.

5.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

5.4 *Timer* (stop-clock)—One that can be read for hours and minutes.

5.5 *Examining Table*—Any elevated surface such as a 3 by 6-ft table with mattress or similar padding to allow the subject to recline.

6. Reagents and Materials

6.1 *Bacteriological Pipette*—10.0 and 2.2-mL or 1.1-mL capacity.[4]

6.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150 to 200-mL capacity and tight closure may be used.[5]

6.3 *Scrubbing Cups*—Sterile glass cylinders, height approximately 2.5 cm, inside diameter of convenient size to place on anatomical area to be sampled. Useful sizes range from approximately 1.5 to 4.0 cm.

6.4 *Rubber Policeman*—Can be fashioned in the laboratory or purchased from most laboratory supply houses.

6.5 *Test Formulation*—With directions for use.

6.6 *Sterile Drape or Dressing*[6]—Used to cover treated skin sites.

6.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isocytlphenoxypolyethoxyethanol[7] in 1 L of distilled water. Include in this formulation an inactivator specific for the antimicrobial in the test formulation. Adjust to

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[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

[3] See *Federal Register*, Vol 46, No. 17, Jan. 27, 1981.

[4] Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

[5] Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

[6] A suitable covering is TELFA Non-Adherent Dressing, No. 3279, from the Kendall Co.; Hospital Products; Boston, MA 02101.

[7] Triton X-100, is available from Rohm and Haas Co., Philadelphia, PA.

pH 7.8. Dispense in 100-mL volumes and sterilize for 20 min at 121°C.

6.8 *Dilution Fluid*—Butterfield's[8] phosphate buffered water adjusted to pH 7.2 and containing an antimicrobial inactivator specific for the test formulation. (See Practices E 1054.)

6.9 *Plating Medium*—Soybean-casein digest agar.[9]

7. Test and Control Skin Sites

7.1 The skin sites selected for use in evaluating the effectiveness of the pre-operative skin preparation should represent body areas that are common surgical sites and should include both dry and moist skin area. The sites should possess bacterial populations large enough to allow demonstrations of bacterial reduction of up to 2.0 $\log_{10}$ cm^2 on dry skin sites and up to 3.0 $\log_{10}$ cm^2 on moist sites. Baseline populations of at least 4.5 $\log_{10}/cm^2$ on wet skin sites and of at least 3.5 $\log_{10}/cm^2$ on dry skin sites are recommended. A suitable dry skin area is the abdomen, and a suitable moist area is the axilla or groin.

7.2 Abdominal treatment areas are to be located within a 5 by 5-in. (12.7 by 12.7-cm) site located in the vicinity below the umbilicus, one either to the right or left of the median. Using a 5 by 5-in. (12.7 by 12.7-cm) sterile paper template, the corners of each site are marked as 1, 2, 3, and 4 directly on the skin, using a sterile surgical skin marker. Numbering is to be the same for all abdominal sites; number 1 is placed at the top corner to the subject's right, and numbers 2, 3, and 4 are assigned in order clockwise from 1. Each quadrant of each site represents a different treatment exposure of either 10 min, 30 min, or ≥4 h. The remaining fourth quadrant is used as a baseline count site.

7.3 Similarly, using a 2 by 5-in. (5.1 by 12.7-cm) sterile template, 2 by 5-in. (5.1 by 12.7-cm) sites are delineated on the inner aspect of both upper thighs within 2 in. and parallel to the leg crease below the groin. The top corners of the sites are numbered beginning on the subject's right lateral with number 1 and in order through 4.

7.3.1 Three 2 by 2-in. (5.1 by 5.1-cm) areas represent different exposures of 10 min, 30 min, or ≥4 h, and the fourth area is used as a baseline site.

7.3.2 The abdominal and groin sites are illustrated in Fig. 1. A panelist may have two treatments applied to his abdomen, provided that a 5 by 5-in. (12.7 by 12.7-cm) site is defined both on the right and left side of the median. Only one treatment, however, may be applied to the groin, as the right and left groin sites are to receive the same treatment.

8. Procedure

8.1 Recruit healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, or other skin disorders which may affect the integrity of the study and in sufficient numbers such that 12 qualified abdominal sites and 12 qualified groin sites are available for treatment. This may require entering more than six volunteers into the study, as in all probability not everyone entered into the study will exhibit

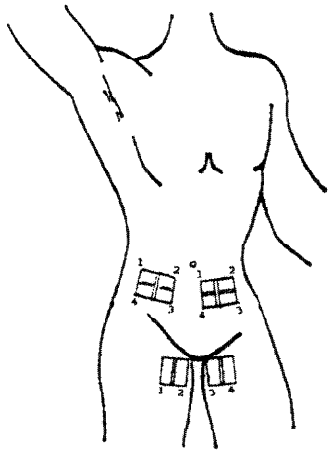

FIG. 1 Illustration of Approximate Location of Test Areas

in the designated skin sites the bacterial population required to demonstrate the log reduction specified in 7.1.

8.2 Instruct the volunteers to avoid contact with antimicrobials (other than the test formulation) for the duration of the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, bathing soaps and body powders. Volunteers also are to refrain from wearing clothes that have been treated with a fabric softener. Bathing in biocide treated pools, hot tubs, spas, and so forth, should be avoided.

8.2.1 Provide volunteers with a kit of non-antimicrobial personal care products for exclusive use during the test. Volunteers are not to shower or tub bathe in the 24-h period prior to the application of test material or microbial sampling. Sponge baths may be taken, but the skin sites to be used in the study are to be excluded.

8.2.2 If the skin sites to be used include areas that would require shaving prior to surgery, for example, the axilla, these sites should be shaved no later than 48 h prior to the application of test formulation or microbial sampling.

8.3 After volunteers have refrained from using antimicrobials for at least 2 weeks, obtain an estimate of baseline bacterial population from a 2 by 2-in. (5.1 by 5.1-cm) area of each 5 by 5-in. (12.7 by 12.7-cm) abdominal site and from a 2 by 2-in. (5.1 by 5.1-cm) area of the left and right groin site. Collect these samples at least 72 h prior to entering subjects into the study. Sampling and enumeration techniques described in Sections 9 and 109 and 10 should be used.

8.4 Based on the initial estimate of baseline bacterial population, select subjects with high abdominal site counts and high groin counts. A total of 12 groin sites and 12 abdominal sites are required.

NOTE 3—A given treatment is to be applied to only one abdominal site on a given subject.

8.5 A second baseline sample is to be collected the day treatment is applied. A single sample is obtained from one 2 by 2-in. (5.1 by 5.1-cm) site in one groin for this baseline estimate and from a 2 by 2-in. (5.1 by 5.1-cm) site within each abdominal area.

8.6 *Treatment Application Procedure*—Immediately after taking the second baseline sample, the treatment is applied

[8] Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.

[9] U.S. Pharmacopeia, XXI: United States Pharmacopeial Convention, Inc., Rockville, MD, see Chapter entitled "Microbial Limits Test," 1985.

according to label directions or as stated in the proposed directions. On the abdomen, the entire 5 by 5-in. (12.7 by 12.7-cm) area that encompasses four 2 by 2-in. (5.1 by 5.1-cm) sites, is prepped. On the groin, the 2 by 5-in. (5.1 by 12.7-cm) area on each thigh that encompasses four 2 by 2-in. (5.1 by 5.1-cm) sites is prepped.

8.7 *Treatment Assignment and Sampling Schedule*—According to a predetermined randomization, a sample of the prepped area is taken from the appropriate site quadrants at 10 min, 30 min, and ≥4 h post treatment using the scrub cup technique (9.1).

NOTE 4—Between the time of treatment application and final sampling, subjects should avoid activities or positions that would cause untreated skin sites to contact treated sites or clothing. To allow the subjects some degree of mobility between the time of treatment and final sampling, the treated skin areas should be draped loosely with a sterile non-occlusive dressing (6.6). This material is applied in such a manner as to protect the treated skin sites from contact with untreated skin.

9. Microbiological Methods

9.1 Quantitative cultures are obtained by the detergent scrub cup technique.[10] Hold a sterile scrubbing cup (6.3) firmly to the skin. Aseptically pipet 2 mL of sterile sampling solution (6.6) into the scrubbing cup and rub the skin with a sterile rubber policeman (6.4) for 1 min using moderate pressure. Aspirate the wash fluid and place in a sterile test tube. Place a second 2-mL aliquot of sampling solution in the scrub cup and rub the skin again for 1 min with the rubber policeman. Pool the two washes and enumerate the bacteria.

10. Enumeration of Bacteria in Sampling Solution

10.1 Enumerate the bacteria in the sampling solution by a standard plate count procedure such as that described in *Standard Methods for the Evaluation of Dairy Products*,[11] but use soybean-casein digest agar (6.9) and a suitable inactivator for the antimicrobial where necessary. Prepare sample dilutions in dilution fluid (6.8). Plate in duplicate. Incubate plated samples from the abdomen at 30° ± 2°C, and those for the groin or axilla at 35 ± 2°C for 48 to 72 h before reading.

11. Determination of Reduction Obtained

11.1 Determine changes from baseline counts obtained with the test material at each sampling interval for each anatomical site.

11.2 For a more realistic appraisal of the activity of products, all raw data should be converted to common (base 10) logarithms. Reduction should be calculated from the average of the logarithms. This will also facilitate statistical analysis of data if desired.

12. Comparison of Test Material With Control Material(s)

12.1 It may be desirable to compare the test material with a placebo or positive control material, or both. If so, the number of test subjects should be increased. The number of extra subjects required will depend upon the number of control post-treatment sampling intervals chosen and the level of statistical significance desired for the test results. Identity of test and control material assignments should be blinded from those counting plates and analyzing data.

12.2 A suggested positive control material is a pre-operative skin preparation approved by the U.S. Food and Drug Administration. A placebo formulation may be the test material without the antimicrobial ingredient.

12.3 Compare, at each sampling interval, changes from baseline counts obtained with the test material to changes obtained with the control materials

13. Precision and Bias

13.1 A precision and bias statement cannot be made for this test method at this time.

14. Keywords

14.1 antimicrobial; efficacy; pre-operative; skin

[10] Williamson, P., and Kligman, A. M., "A New Method for the Quantitative Investigation of Cutaneous Bacteria," *Journal of Investigative Dermatology*, Vol 46, pp. 198–503.

[11] *Standard Method for the Examination of Dairy Products*, Chapter: Standard Plate Count Method, 14th ed., American Public Health Association, Inc., Washington, DC, 1978.

Title:
Quantitative Assessment of Benefits from Using Topical Antimicrobial Hand Products: Case Study on *Salmonella* Risk from Handling Raw Chicken

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Abstract (1850 characters - excluding spaces):

Background:

When preparing chicken during food preparation there is a chance for *Salmonella* and other bacteria to be transferred from the chicken to a subject's hands and subsequently from hands to mouth where infection may occur. Using antimicrobial soap after preparing raw chicken can reduce the probability of infection. A Quantitative Microbial Risk Assessment (QMRA) model was constructed in order to access the probability of infection after preparing raw chicken under two different scenarios.

Methods:

This model was constructed in Microsoft Excel and evaluated using an add in statistical software package Crystal Ball which uses Monte Carlo analysis. The beta-Poisson model was used to calculate the probability of infection for two different scenarios. The subject either washed hands with antimicrobial soap or did not wash their hands at all after handling raw chicken. Specific parameters in the model (i.e. % transfer, $\log_{10}$ reduction, etc.) were described by statistical distributions. Five simulations were run for each scenario by varying the active ingredient or the statistical distribution representing the active ingredient. Each simulation produced 500 probability of infection estimates. A sensitivity analysis was also performed to evaluate which parameters in the model were most sensitive.

Results:

Using antimicrobial soap after handling raw chicken reduces the probability of infection by 3 to 5 orders of magnitude when compared to the no hand washing scenario. Alcohol is a more effective active ingredient than chlorhexidine. The probability of infection was reduced by 2 orders of magnitude when alcohol was used as the active ingredient over chlorhexidine. The most sensitive parameter in the model was the occurrence of *Salmonella* on raw chicken followed by $\log_{10}$ reduction.

Conclusion:

The probability of infection is greatly reduced by the use of antimicrobial soap when handling raw chicken during food preparation. The QMRA model for this analysis is a useful tool for predicting the probability of infection quantitatively. QMRA is an effective method for determining the benefit of topical antimicrobial products.

Topic:
Y01 Public Health

Keywords (Up to three):
Quantitative Microbial Risk Assessment (QMRA), *Salmonella*, Antimicrobial Soap

Designation: E 1174 – 00

Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations[1]

This standard is issued under the fixed designation E 1174; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to determine the effectiveness of antimicrobial handwashing agents for the reduction of transient microbial flora when used in a handwashing procedure.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 In this test method metric units are used for all applications, except for distance in which case inches are used and metric units follow in parentheses.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements see Note 1.

1.5 This method may be used to evaluate topical antimicrobial handwash formulations.

1.6 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[2]

2. Referenced Documents

2.1 *ASTM Standards:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *test organism*—an applied inoculum of an organism that has characteristics which allow it to be readily identified. The test organism is used to simulate a transient topical microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

3.1.2 *resident microorganisms*—microorganisms that live and multiply on the skin, forming a permanent population.

3.1.3 *transient microorganisms*—organisms from the environment that contaminate but do not normally colonize the skin.

3.1.4 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.1.5 *test formulation*—a formulation which incorporates antimicrobial ingredient(s).

3.1.6 *neutralization*—a process which results in quenching the antimicrobial activity of a test material. This may be achieved through dilution of the test material(s) to reduce the antimicrobial activity, or through the use of chemical agents, called neutralizers, to eliminate antibacterial activity.

3.1.7 *cleansing wash*—a non-antimicrobial wash intended to remove gross soil or residues from the hands of the panelists prior to the conduct of the study and as noted throughout the study. This may also be referred to as a cosmetic wash.

3.1.8 *healthcare personnel handwash*—a cleanser or waterless agent intended to reduce transient bacteria on the hands.

4. Summary of Test Method

4.1 This test method is conducted on a group of volunteer panelists who have refrained from using topical antimicrobial formulations for at least one week prior to the initiation of the test. Activity of the test material is measured by comparing the number of test organisms recovered from artificially contaminated hands after use of a handwashing formulation to the number recovered from contaminated hands not exposed to the test formulation. The method describes specific procedures to be followed using *Serratia marcescens* as the test organism. The activity of the test material may be measured following a single wash and multiple washes in a single clay using a neutralization recovery method.

4.2 An alternative test organism is *Escherichia coli*. Culture media and incubation conditions appropriate for this organism should be employed. The investigator should also be aware that there may be health risks associated with the use of this organism and precautions similar to those referenced in Note 1 should be undertaken.

5. Significance and Use

5.1 The procedure may be used to test the effectiveness of antimicrobial handwashing agents. The test formulations may be designed for frequent use to reduce the transient bacterial flora on hands.

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[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E35.15 Antimicrobial Agents.

Current edition approved August 10, 2000. Published November 2000. Originally published as E 1174 – 87. Last previous edition E 1174 – 94.

[2] *Federal Register*, Vol 46, No. 17, Jan. 27, 1991.

[3] *Annual Book of ASTM Standards*, Vol 11.04.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter.

6.2 *Incubator*—Any incubator capable of maintaining the following temperatures: *S. marcescens* (25 ± 2°C) or *E. coli* (35 ± 2°C). This temperature is required to ensure pigment production for *S. marcescens*.

6.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

6.4 *Timer* (Stop-clock)—One that can be read for minutes and seconds.

6.5 *Handwashing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists.

6.5.1 *Water faucet(s)*—To be located above the sink at a height which permits the hands to be held higher than the elbow during the washing procedure.

6.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature of 40 ± 2°C.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity.[4]

7.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150–200 mL capacity and tight closures may be used.[5]

7.3 *Erlenmeyer Flask*—2-L capacity for culturing test organism.

7.4 *Cleansing Wash*—A mild, non-antimicrobial solid or liquid soap. (The investigator may choose to use the product vehicle.)

7.5 *Test Material*—Directions for use of the test material may be utilized. If directions are not available, use directions provided in this test method.

7.6 *Gloves*—Loose-fitting, unlined, powder-free gloves which possess no antimicrobial properties, or equivalent.[6] (Plastic bags with low bioburden may be used in place of gloves.)

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isooctylphenoxypolyethoxyethanol[7] and with appropriately validated neutralizers in 1-L distilled water. Adjust pH to 7.8 with 0.1 *N* HCl or 0.1 *N* NaOH. Dispense so that final volume after sterilization is 75 ml, sterilized at 121°C.[8]

7.8 *Dilution Fluid*—Sterile Butterfield's Buffer[9] or other suitable diluent, adjusted to pH 7.2 with effective neutralizer for the test material. Adjust pH with 0.1 *N* HCl or 0.1 *N* NaOH. See Test Methods E 1054.

7.9 *Agar*—Soybean-casein digest agar, or other solid media appropriately validated to support growth of the test organism with appropriate neutralizers if needed.

7.10 *Broth*—Soybean-casein digest broth or other liquid media appropriate to support growth of the test organism.

8. Test Organism

8.1 *Serratia marcescens* (ATCC 14756) is to be used as the test organism. This is a strain having stable pigmentation at 25°C.

8.2 *Escherichia coli* (ATCC 11229) is an alternative test organism. When *E. coli* is used, the plating agar should include a suitable indicator (e.g. MUG[10]).

NOTE 1—**Warning:** The application of microorganisms to the skin may involve a health risk. Prior to applying the test organism to the skin, the antibiotic sensitivity profile of the strain should be determined. If the strain is not susceptible to gentamicin, do not use it. If an infection occurs, the antibiotic sensitivity profile should be made available to the attending clinician.

Following the subject's last contamination and wash with the formulation, the subject's hands are to be sanitized by scrubbing with 70% isopropanol solution or equivalent. The purpose of this alcohol scrub is to destroy residual test organisms on the skin.

8.3 *Preparation of Test Organism Suspension*

8.3.1 *S. marcescens*—A homogeneous culture is used to inoculate the hands. The stock culture should be at least two 24 hour broth transfers from the original ATCC culture, but there should be no more than 5 transfers removed from the ATCC culture. From the stock culture of *Serratia marcescens* (ATCC 14756) inoculate the appropriate volume of soybean-casein digest broth (7.10) with 0.1 milliliter of stock culture of *S. marcescens*/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at 25°C ± 2°C. Broth should develop a red pigment.

8.3.2 *E. coli*—A homogeneous culture is used to inoculate the hands, the stock culture should be at least two 24 hour broth transfers from the original ATCC culture, but no more than 5 transfers removed from the ATCC culture. From the stock culture of *Escherichia coli* (ATCC 11229) inoculate the appropriate volume of soybean-casein digest broth (7.10) with 0.1 milliliter of stock culture/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 hours at 35 ± 2°C.

8.4 Swirl or shake suspension before the withdrawal of each aliquot. Assay the suspension for number of organisms at the beginning and end of the use period. Do not use a suspension for more than 8 hours. The suspension may not vary more than ± 0.5 $\log_{10}$ cfu/mL over an 8 hour period.

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[4] Presterilized/disposable bacteriological pipettes are available from most local laboratory supply houses.

[5] Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

[6] A suitable glove would be Pharmaseal 8873C, (sterile) Flexam Latex Procedure Glove from American Pharmaseal Laboratories, Glendale, CA 91209. A zone of inhibition test such as AATCC Test Method 90-1965 may be used to evaluate antimicrobial properties of gloves, *AATCC Test Methods*, American Association of Textile Chemists and Colorist, 1968 Technical Manual, Section B-75.

[7] Triton X-100, Rohm and Haas Co., Philadelphia, PA.

[8] Peterson, A.F., "The Microbiology of the Hands: Evaluating the Effects of the Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130, 1973.

[9] Horowitz, W. (Ed.) 1980. *Official Methods of Analysis of the AOAC*, 13th Ed., Sec. 46.013 (m), p. 825. Assoc. of Off. Anal. Chemists, Washington, D.C. 1018 pp.

[10] *United States Pharmacopeia XXII*: United States Pharmacopeial Convention, Inc., Rockville, MD, Chapter entitled "Microbial Limits Test." The MUG (4-methylumbelliferyl-β-D-gluconride) substrate is hydrolyzed by β-D-gluconridase to yield a fluorescent end product, 4-methylumbelliferone. β-D-gluconridase is possessed by *E. coli* (ATCC 11229). MUG is incorporated into the appropriate growth medium at 0.05 grams/L.

9. Subjects

9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, hangnails, or other skin disorders.

9.2 Instruct subjects to avoid contact with antimicrobial products (other than the test material as dispensed for each test wash) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions and soaps, also such materials as acids, bases and solvents. Bathing in biocide treated pools, hot tubs, or spas should be avoided. Subjects are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.

10. Procedure

10.1 After subjects have refrained from using antimicrobial formulations for at least 7 days, they perform a 30 second cleansing wash (7.4) in the same manner that is described for the test and control formulations. This procedure removes oil and dirt and familiarizes the panelists with the washing technique.

10.2 *Hand Contamination*—A liquid suspension of the test organism containing a minimum of 1×10^8 cfu/mL is used. See Table 1.

10.2.1 A 1.5mL aliquot of the test organism suspension is dispensed into the subjects' cupped hands. This aliquot is rubbed over the entire surfaces of the hands for 20 ± 5 s (front and back) not reaching above the wrist. The hands are then held motionless away from the body and allowed to air dry for approximately 30 ± 5 s.

TABLE 1 Hand Contamination with Test Organism Suspension

Volume	Spread Time	Dry Time
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	90 sec

10.2.2 To continue the contamination of the hands, an additional 1.5mL aliquot of the test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 seconds, and air dried for 30 ± 5 seconds.

10.2.3 To complete the contamination, a final 1.5mL aliquot of test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 seconds, and air dried for 90 ± 5 seconds (Table 1).

NOTE 2—The hands may still be wet after the 90 seconds.

10.2.4 The total test organism suspension applied to the hands is 4.5 mL. Contamination may take approximately 5 minutes. This method of contamination minimizes the loss of test organism while spreading.

10.3 *Contamination Schedule*—The subjects' hands are contaminated with the test organism prior to the baseline bacterial sample collection and prior to each washing with the test material. Table 2 below illustrates a typical test. The number of repeated test washes may be reduced or eliminated at the discretion of the investigator.

TABLE 2 Hand Contamination and Recovery Schedule

Name	Contamination	Type of Wash	Recovery
Cleansing Wash	no	Cleansing Wash	no
Baseline	yes	no	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	no	Cleansing Wash	no
Test Wash 1	yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	no	Cleansing Wash	no
Test Wash 2–10	yes	Test Formulation	no
Test Wash 11	yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer

10.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of marker organisms surviving on the hands. Bacterial sampling will follow the procedures outlined in Section 12.

11. Wash and Rinse Procedure

11.1 Conduct the test in accordance with the use directions for the test material. If test material directions are not available, the wash and rinse procedure described as follows should be used. Table 2 above shows the contamination and recovery schedule for the overall study.

11.2 *Liquid Formulations*

11.2.1 Dispense 5 ml of test material into cupped hands. Spread over hands and lower ⅓of forearms.

NOTE 3—The 5 ml volume has been chosen for test purposes due to the requirement for washing hands and forearms.

11.2.2 Sparingly wet contaminated hands with 40 ± 2°C tap water.

11.2.3 Wash in a vigorous manner for 30 ± 5 seconds all surfaces of the hands and the lower third of the forearm. Caution should be exercised to retain the test material in the hands. If the lather becomes too dry, a small amount of water may be added to maintain lather.

11.2.4 Rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 seconds. Caution should be exercised to avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

11.2.5 Subject's hands and forearms are lightly patted dry with paper toweling.

NOTE 4—After washes requiring sampling, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

11.3 *Waterless Formulations*[11]

11.3.1 Dispense 5 mL of test material into cupped hands.

NOTE 5—The 5 ml volume has been chosen for test purposes due to the requirement for washing hands and forearms.

11.3.2 Distribute test material over all surfaces of the hands and the lower third of the forearms. Continue rubbing in a

[11] An alternative test methodology may be found in European Standard CEN-1500: Chemical Disinfectants and Antiseptics - Hygenic Handrub - Test Method and Requirements (phase2/step2), July, 1997.

vigorous manner for 30 ± 5 seconds or until dry. Caution should be exercised to retain the test material in the hands.

11.3.3 Subject's hands may be held upright and motionless prior to Bacterial Recovery (Section 12).

11.4 *Solid Formulations*

11.4.1 Sparingly wet contaminated hands and forearms with 40 ± 2°C tap water.

11.4.2 Wet the product.

11.4.3 Rub the product between the hands and on the forearms for 15 ± 3 seconds. Place product aside.

11.4.4 Lather lower third of forearms and hands for an additional 30 ± 5 seconds. If the lather becomes too dry, a small amount of water may be added to maintain lather.

11.4.5 Rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 seconds. Caution should be exercised to avoid contact with the sink and fixtures to eliminate contamination from the sink surfaces.

11.4.6 Subject's hands and forearms are lightly patted dry with paper toweling.

11.5 *Other Product Forms*

11.5.1 Use standardized amount (e.g. weight, volume) of test material in accordance with use directions.

11.6 After washes requiring sampling, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

12. Bacterial Recovery

12.1 Within 5 minutes after specified washes (10.3), place gloves (7.6) used for sampling on the hands. Add 75 mL of sampling solution (7.7) with neutralizer to each glove and secure gloves above the wrist.

12.2 Uniformly massage all surfaces of the hand for 1 min ± 5 seconds.

12.3 Aseptically retrieve a 3-5 mL sample of the fluid in the glove by pulling the glove away from the wrist, inserting a pipet into the finger region of the glove, and withdrawing the fluid.

12.4 The dilution and plating of the recovered sampling solution is completed within 30 minutes after sampling.

13. Enumeration of Bacteria in Sampling Solution

13.1 *S. marcescens*

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (12.3) using standard microbiological techniques, such as membrane filtration or spread plating. The pour plate technique is not recommended because subsurface *S. marcescens* colony forming units may not exhibit the red pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator as recovery medium.

13.1.3 Incubate prepared plates 48 ± 4 h at 25 ± 2°C. Standard plate counting procedures are used to count only the red pigmented *S. marcescens*.

13.2 *E. coli*

13.2.1 Enumerate the *E. coli* in the sampling solution using standard microbiological techniques, such as membrane filtration, pour or spread plating. Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator and indicator (MUG^{10}) as recovery medium.

13.2.2 Incubate prepared plates 48 ± 4 hour at 35 ± 2°C. Standard plate counting procedures are used to count only the fluorescent (MUG^{10}) *E. coli* colonies. Fluorescent colonies are counted using long-wave UV light.

14. Determination of Reduction

14.1 Convert plate counts (cfu/hand) to $\log_{10}$. Average left and right hands.

14.2 Determine Log_{10} Reductions at each recovery interval/wash using the following formula:

$$\text{Log}_{10}\ \textit{Reduction at Sampling Interval} = \text{Log}_{10}\ \textit{Baseline Recovery} - \text{Log}_{10}\ \textit{Sampling Interval} \quad (1)$$

15. Comparison of Test Material

15.1 It may be desirable to compare the test material with other test formulations. If this is the case, an equivalent number of panelists should be assigned to each formulation on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. Both products should be run concurrently.

16. Precision and Bias

16.1 A precision and bias statement cannot be made for this test method at this time.

17. Keywords

17.1 antimicrobial; contaminant; efficacy; handwash; healthcare; marker organism; simulant

Designation: E 1174 - 87

Standard Test Method for Evaluation of Health Care Personnel Handwash Formulation[1]

This standard is issued under the fixed designation E 1174; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to determine the ability of an antimicrobial handwashing agent to give reduction of transient microbial flora (contaminants) when used in a hand washing procedure.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 *This standard may involve hazardous materials, operations, and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements see Notes 1 and 2.

2. Referenced Document

2.1 *ASTM Standard:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[2]

3. Summary of Test Method

3.1 This test method is conducted on a group of volunteer panelists who have refrained from using topical antimicrobials for at least one week prior to the initiation of the test. Activity is measured by comparing the number of a marker bacteria recovered from artificially contaminated hands after use of the handwashing formulation to the number recovered from contaminated unwashed hands. A broth culture of *Serratia marcescens*, a species of bacteria which produces a red pigment color on an agar surface is used as the contaminant bacteria. The activity of the formulation is measured following 1, 3, 5, and 7 handwashings.

4. Significance and Use

4.1 The procedure should be used to test the degerming effectiveness of antimicrobial hand washing agents, used by health care personnel, that are intended for frequent use and that are intended to reduce the level of contamination acquired through contact with contaminated objects or people.

4.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[3]

5. Apparatus

5.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter.

5.2 *Incubator*—Any incubator capable of maintaining a temperature of 25 ± 2°C may be used. This temperature is required to assure pigment production by the *Serratia*.

5.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

5.4 *Timer* (Stop-clock)—One that can be read for minutes and seconds.

5.5 *Handwashing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists.

5.5.1 Water faucet(s) to be located above the sink at a height which permits the hands to be held higher than the elbow during the washing procedure.

5.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature of 40 ± 2°C.

6. Materials and Reagents

6.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity.[4]

6.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150–200 mL capacity and tight closures may be used.[5]

6.3 *Erlenmeyer Flask*—2-L capacity for culturing test organism.

6.4 *Baseline Control Soap*, a liquid castile soap or other liquid soap containing no antimicrobial.

6.5 *Test Formulation*—Directions for use of test formulation should be included if available. If there are not any available, use directions provided in this test method (see 9.5).

6.6 *Gloves*—Loose-fitting gloves of latex, unlined, possessing non-antimicrobial properties.[6]

6.7 *Sampling Solution*[7]—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isooctylphenoxypolyethoxyethanol[8] in

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[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 Antimicrobial Agents.

Current edition approved July 31, 1987. Published September 1987.

[2] *Annual Book of ASTM Standards*, Vol 11.04.

[3] See *Federal Register*, Vol 46, No. 17, Jan. 27, 1981.

[4] Presterilized/disposable bacteriological pipettes are available from most local laboratory supply houses.

[5] Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

[6] A suitable glove Pharmaseal 8873C, (sterile) Flexam Latex Procedure Glove from American Pharmaseal Laboratories, Glendale, CA 91209. A zone of inhibition test such as AATCC Test Method 90-1965 may be used to evaluate antimicrobial properties of gloves. *AATCC Test Methods*, American Association of Textile Chemists and Colorist, 1968 Technical Manual, Section B-175.

[7] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130, 1973.

[8] Triton X-100, Rohm and Haas Co., Philadelphia, PA.

1-L distilled water. Adjust pH to 7.8 with 0.1 *N* HCl or 0.1 *N* NaOH. Dispense in 75-mL volumes and sterilize for 20 min 121°C.

6.8 *Dilution Fluid*—Butterfields sterile phosphate buffered water[9] adjusted to pH 7.2 with suitable inactivator for the antimicrobial. Adjust pH with 0.1 *N* HCl or 0.1 *N* NaOH. (See Test Methods E 1054.)

6.9 *Agar*—Contains Soybean-casein digest agar[10] plus suitable inactivator.

6.10 *Broth*—Soybean-casein digest: 1000 mL per 2-L flask.

7. Test Organism

7.1 *Serratia marcescens* ATCC No. 14756 is to be used as a marker organism. This is a strain having stable pigmentation.

NOTE 1: **Warning**—The application of microorganisms to the skin may involve a health risk. Prior to applying the *S. marcescens* strain to the skin, the antibiotic sensitivity profile of the strain should be determined. If the strain is not sensitive to Gentamycin, do not use it. If an infection occurs, the antibiotic sensitivity profile should be made available to the attending clinician.

Following the panelist last contamination and wash with the test formulation, the panelist's hands are to be sanitized by scrubbing with a 70 % ethanol solution. The purpose of this alcohol scrub is to destroy any residual *S. marcescens*.

7.2 *Preparation of Marker Culture Suspension*—From stock culture inoculate *Serratia marcescens* ATCC No. 14756 in 2-L flask containing 1000 mL of Soybean-casein digest broth (6.10). Incubate for 24 ± 4 h at 35°C. Stir or shake the suspension before each aliquot withdrawal. Assay suspension for number of organisms by membrane filtration technique or surface inoculation at the beginning and end of the use period. Do not use a suspension for more than 8 h.

8. Panelists

8.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, hangnail or other skin disorders that may affect the integrity of the test and such that 12 subjects complete the study.

8.2 Instruct the volunteers to avoid contact with antimicrobials (other than the test formulation) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions and soaps, also such materials as acids, bases and solvents. Bathing in biocide treated pools, hot tubs, spas, etc., should be avoided. Volunteers are to be provided with a kit of non-antimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobials cannot be avoided.

9. Procedure

9.1 After panelists have refrained from using antimicrobials for at least 7 days, they perform a 30-s practice wash in the same manner that is described for the test and control formulations, except that a solution of non-antimicrobial bland soap (see 6.4) is used. This procedure removes oil and dirt and familiarizes the panelists with the washing technique.

9.2 *Contaminant Suspension and Hand Contamination*—The contaminant is a liquid suspension of *Serratia marcescens* containing at least 10 organisms per mL. (See Test Methods E 1054.) Five millilitres of the contaminant culture are dispensed onto the hands then rubbed over the surfaces of the hands, not reaching above the wrist. Application and spreading should involve about 45 s. The hands are then held still away from the body and allowed to air dry for 1 min.

9.3 *Contamination Schedule*—The panelist hands are contaminated with the marker organism according to the following schedule:

9.3.1 Prior to the baseline bacterial sample collection. (See 9.4.)

9.3.2 Prior to the 1st, 3rd, 5th, and 7th washes with the test material.

9.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of marker organisms surviving on the hands. Bacterial sampling will follow the procedures outlined in section 9.6.

9.5 *Wash and Rinse Procedure*—The wash and rinse procedure described as follows is for all washes with the test formulation whether or not they are preceded by artificial contamination of the hands. Five millilitres or an amount specified by the manufacturer of the test formulation is dispensed onto the hands and rubbed over all surfaces, taking caution not to lose or dilute the substance. After the material is spread, a small amount of water is added from the tap and the hands are completely lathered for 30 s. The lower third of the forearm is also washed. After completion of the wash, hands and forearms are rinsed under tap water at 40 ± 2°C for 30 s. A total of seven washes with the test formulation are involved. Bacterial samples are taken following the first, third, fifth, and seventh washes.

9.6 *Bacterial Sampling*—After specified washes, place rubber gloves (6.6) used for sampling on the right and left hand. Add 75 mL of sampling solution (6.7) to each glove and secure gloves above the wrist. After adding sampling solution, uniformly massage all surfaces of the hand for 1 min. After massaging aseptically sample the fluid of the glove.

NOTE 2: **Caution**—No neutralizer for the antimicrobial in the handwash formulation is included in the sampling solution to inhibit the antimicrobial action once sampling is initiated. The 75 mL of sampling fluid may be sufficient to dilute out the activity of antimicrobial, however, this should be demonstrated using a procedure such as described in Test Methods E 1054.

If neutralization is not accomplished by dilution include an antimicrobial inactivator specific for the test formulation being evaluated in the sampling solution used to collect the bacterial samples from the hand following the final wash with test formulation.

A definite recommendation regarding the inclusion of an inactivator in sampling solution (6.7) used for bacterial collection prior to the final wash cannot be made. Two points should be considered in making a decision: (*1*) If an inactivator is included in all sampling fluid, will residual inactivator on the skin reduce the efficacy of the test formulation in subsequent washes and result in higher than expected bacterial counts? and (*2*) Can samples collected without an inactivator be

[9] Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.

[10] *United States Pharmacopeia*, XX: United States Pharmacopeial Convention, Inc., Rockville, MD, Chapter entitled "Microbial Limits Test."

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processed quickly enough to avoid decreased bacterial counts due to continued action of the test formulation? Whatever the decision, to facilitate the comparison of results across studies, the investigator should indicate whether or not an inactivator has been included.

10. Enumeration of Bacteria in Sampling Solution

10.1 Enumerate the *S. marcescens* in the sampling solution using standard microbiological techniques, such as membrane filter technique or surface inoculation technique. Prepare sample dilutions in dilution fluid (6.8). Use soybean-casein digest agar with suitable inactivator as recovery medium. Incubate prepared plates 48 h at 25 ± 2°C. Standard plate counting procedures are used to count only the red pigmented *S. marcescens*.

11. Determination of Reduction

11.1 Determine at each sampling interval changes from baseline counts obtained with test material.

12. Comparison of Test Material with a Control Material

12.1 It may be desirable to compare the test material with a control material. If this is the case, an equivalent number of panelists should be assigned to the control product on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. Both products should be run concurrently.

12.2 Compare, at each sampling interval, changes from baseline counts obtained with test material to changes obtained with control material.

13. Precision and Bias

13.1 A precision and bias statement cannot be made for this test method at this time.